



RESEARCH ARTICLE

Growth dynamics of *Trichoderma asperellum*: Interactive effects of temperature and sugar concentration

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Abstract

Trichoderma species are ubiquitous soil-dwelling fungi that are often linked to decomposing plant waste. Their potential as effective biocontrol agents in sustainable agriculture are widely recognised. The development of these fungi is strongly influenced by environmental factors such as temperature and the availability of nutrients. In this study 4 native isolates of *Trichoderma asperellum* were collected from the Department of Plant Pathology, College of Agriculture, Central Agricultural University, Imphal during season 2021–22. These isolates were evaluated *in vitro* to ascertain the impact of temperature and sugar concentration on mycelial growth. While pure cultures were maintained on potato dextrose agar (PDA), growth characteristics such as radial growth, fresh weight and dry weight of the mycelium were measured. The findings demonstrated that radial growth gradually increased as temperatures rose from 30 to 34 °C and sugar concentrations ranged from 15 to 30 g L⁻¹, with 30 °C showing the best radial growth. In media supplemented with sugar concentrations ranging from 10 to 25 g L⁻¹, the maximum fresh and dry mycelial weights were measured at 26 °C and 30 °C respectively. Sugar concentration alone showed no significant effect on mycelial biomass, but when combined with temperature, it significantly altered the growth response. Overall, the study demonstrates that sugar concentration and temperature, both separately and in combination, are critical in controlling the growth dynamics of *T. asperellum* isolates, offering important information for maximising their mass multiplication and field application as a biocontrol agent.

Keywords: biocontrol agents; environmental factors; mycelial growth; potato dextrose agar; *Trichoderma asperellum*

Introduction

The name *Trichoderma* is derived from the Greek words thrix (hair) and derma (skin) and was first coined by Persoon, a mycologist in 1794 (1). These are asexually reproducing and free-living filamentous fungi. Due to its widespread distribution, highly opportunistic invader, avirulent plant symbionts, food competition and mycoparasitism, it is a good biocontrol agent. These characteristics make this fungus widespread and environmentally sound (2, 3). These fungi are characterised by their rapid growth and characteristic colonies, which are often present as tufts or pustules.

Their conidiophores are repeatedly branched and bear large, flask-shaped (lageniform) phialides that produce hyaline to green conidia in slimy masses. Some species may also form chlamydospores (4). *Trichoderma* species are globally distributed and occur in nearly all soil types, including those in forest humus layers (5). In agricultural crop production, *Trichoderma* is employed for several purposes. Although it mostly interacts with pathogenic fungus, *Trichoderma* also interacts with other microbes (6, 7). Hyperparasitism, competition and antibiosis are some of these interactions. Other fungi activity was inhibited by competition for food, nutrients and space by environmental modification (8).

By fostering a symbiotic relationship with plants and releasing a variety of secondary metabolites, such as growth hormones, endochitinase and proteolytic enzymes, *Trichoderma* enhances the general health of plants by utilising plant-microbe interactions (8). The number of beneficial microorganisms for plants in the soil is increased by the biofertiliser, which is also employed as a soil conditioner. It aids in greenhouse gases reduction, which are major contributors to global warming, such as carbon dioxide and methane (9). It has been reported that the chemical pollutants in the soil can be broken down by using *Trichoderma* and other microorganisms. Innovative technologies that have the potential to reduce a variety of soil pollution issues include bioremediation and phytoremediation in conjunction with microbes. The genus *Trichoderma* is genetically diverse and has a range of capabilities among different strains that are important for agriculture and industry (10). The potential *Trichoderma* improves the physical and chemical characteristics of soil by acting on chemicals and metal contaminants through the activity of different enzymes (11, 12). *Trichoderma* spp. has been found to resist and accumulate heavy metal pollutants like nickel (Ni), cadmium (Cd), zinc (Zn), lead (Pb) and arsenic (As) (10, 11).

The application of agrochemicals in intensive agriculture has resulted in the accumulation of pollutants and the deterioration of crop performance and soil quality. Inoculating soil with *Trichoderma* has been shown to break down chemical pollutants and release nutrients from those agrochemicals for plants (11). Many studies have shown that *Trichoderma* is an efficient biocontrol agent for phytopathogenic bacteria since Weindling first identified the genus *Trichoderma* spp. as a biocontrol agent in 1930 (13). Additionally, it has been shown that the fungi improve plants defence mechanisms. Therefore, using *Trichoderma* as an effective biocontrol agent will undoubtedly guarantee long-term disease management.

Trichoderma species are fast-growing fungus with a wide range of substrate use and persistent conidia. In terms of living space and nourishment, they are fierce rivals (14). Moreover, *Trichoderma* strains have inherent resistance to various toxic compounds like fungicides, herbicides and phenolic compounds. Competing for limited nutrients is very important in the biocontrol of phytopathogens because starvation is the most common cause of death for microorganisms. Filamentous fungi can absorb iron and when these fungi are starved of iron, they secrete siderophores, which are low-molecular-weight ferric-iron-specific chelators. Very potent siderophores that chelate iron and inhibit the growth of other fungi are secreted by *Trichoderma* strains (8). Therefore, the biocontrol function of *Trichoderma* is affected by the nature of the soil. Bio-control agents are the best alternative to chemicals for the successful management of plant diseases. Because it can inhibit soil-borne plant pathogens, *Trichoderma* is frequently employed as a biological control agent in plant disease management (15).

Trichoderma, a biological fungus widely used for plant pest control, mainly exists in the soil, air, plant surface and other ecological environments and can effectively control a variety of plant diseases (16–18). *Trichoderma* is mainly used to treat soil-borne diseases and some leaf and panicle diseases of various plants.

Trichoderma can improve the environment for agrochemical pollution, promote plant development, boost plant resistance and enhance nutrient consumption efficiency in addition to preventing disease. For several crop species, *Trichoderma* spp. serves as a biocontrol agent that is safe, economical, effective and safe for the environment (19).

Recently studies showed that by promoting plant growth, development and resistance, the fungi act as biocontrol agents and boost agricultural yields. Biocontrol techniques that also induce defensive or systemic resistance reactions in plants include mycoparasitism, antibiotics and resource competition. The *Trichoderma* biocontrol program depends on these reactions. *Trichoderma* species have been extensively employed in agricultural applications due to their well-known biological control mechanism (14). Biological control offers a promising and sustainable solution for managing plant diseases and has been the focus of extensive research. Advancing the use of biocontrol methods involves not only the genetic enhancement of microbial antagonists but also the optimisation of their production and delivery systems (20). Among these, *Trichoderma* species stand out as highly effective mycoparasites. When used in combination with lower doses of chemical fungicides, *Trichoderma* can suppress plant pathogens with reduced ecological impact (21). Recognised for their cost-effectiveness, environmental safety and broad-spectrum antagonistic activity, *Trichoderma* spp. and other biocontrol agents are increasingly favoured over synthetic chemicals. Given the rising costs and harmful consequences associated with chemical pesticides, the adoption of biological control strategies represents a viable and eco-friendly alternative that is gaining global acceptance (22). One of the advantageous microorganisms in the agro ecosystem that affects crop productivity and soil health is *Trichoderma*. It is more dependable for usage in agriculture because of its antagonistic properties with plant harmful microorganisms. However, its use is not limited to its anti-pathogenic effect, but it is also used as a biofertiliser, plant growth stimulator, bioremediation agent and biological as well as economic crop production enhancer. Since *Trichoderma* holds promise for the reduction of dangerous pesticides in agriculture, its use should be promoted (14).

Climate change has raised the average global temperature causing the necessity of the knowledge of the impact of the temperature changes on fungi. Climate change is a significant factor that is affected by temperature, which is a significant abiotic factor. The species of *Trichoderma asperellum* exhibit different reaction towards the various environmental factors such as pH, temperature and sugar concentration. The research was therefore carried out to find the interactive impact of temperature and sugar concentration on the growth of *T. asperellum* isolates.

Materials and Methods

Four isolates of *Trichoderma* were collected (Table 1), from the Department of Plant Pathology, College of Agriculture, Central Agricultural University, Imphal. On potato dextrose agar (PDA) each of the *T. asperellum* isolates was kept in the form of pure culture.

Table 1. List of the isolates of *Trichoderma asperellum* were used for the study and their accession number

Sl. No.	Biocontrol agents	Isolation code	Accession No.
1	<i>Trichoderma asperellum</i> (TA1)	TKS-H	MH257327
2	<i>Trichoderma asperellum</i> (TA2)	NCIPMCAU-7	KU933475
3	<i>Trichoderma asperellum</i> (TA3)	NCIPMCAU-25	KT601340
4	<i>Trichoderma asperellum</i> (TA4)	NCIPMCAU-123	KU933476

Subculturing of isolates was done on PDA medium on Petri plates (90 mm in diameter) for assessment of growth attributes.

Effect of interaction between temperature and sugar concentration on radial growth, fresh weight and dry weight of *Trichoderma asperellum*

The effect of interaction between temperature and sugar concentration on radial growth, fresh weight and dry weight of *T. asperellum*. Potato dextrose agar and potato dextrose broth (PDB) respectively were used to ascertain the effect of temperature and sugar level on the radial growth, fresh weight and dry weight of *T. asperellum*. Five (250 mL) conical flasks were prepared with 150 mL of medium in them. Sterilisation of these flasks was done in an autoclave at 1.1 kg cm⁻² for 20 min. The level of sugar concentration (10 g, 15 g, 20 g, 25 g and 30 g/L) was used and 20 mL of the sterilised PDA was put into Petri plates that were left to solidify. The mycelial discs (5 mm in diameter) were cut out of the actively growing cultures of fungi with a cork borer and put in the middle of the plates. All the treatments were replicated five times and experimented in five temperature conditions (26, 28, 30, 32 and 34 °C). The plates inoculated were incubated at respective temperatures until all the fungi were covered and radial growth (in cm) was noted after every 24 hr.

A 50 mL of broth containing different sugar concentrations (10, 15, 20, 25 and 30 g/L) was poured into 100 mL conical flasks, sterilised at 1.1 kg cm⁻² for 20 min and then cooled. Active cultures were presented in the form of 5 mm mycelial discs that were placed in flasks. The treatments were repeated 5 times and performed at five temperatures (26, 28, 30, 32 and 34 °C). The fungal cultures in the flasks were incubated in their respective temperatures of 5 days. The fungal mycelia after incubation were filtered on pre-weighed Whatman No.1 filter paper and dried overnight in a hot air oven at 60 °C. The result of subtracting the weight of the filter paper to the total weight after drying was the dry weight of the fungal biomass. The final dry weight of fungal growth was determined using the formula:

$$W = W_2 - W_1 \quad (\text{Eq. 1})$$

Where, W = Weight of the mycelial mat

W₁ = Weight of the filter paper

W₂ = Total weight of the fungal mycelial mat and filter paper

Statistical analysis

The experimental data collected were analysed statistically wherever necessary. Analysis of variance (ANOVA) was performed considering the interaction between factors. In this study, a three-factor factorial randomised complete block design (FRCBD) was employed to evaluate the effects of selected levels of three factors along with 4 fungal isolates. The isolates were consistently used across all combinations of the physical factors tested, which included temperature and sugar concentration. Statistical analysis was conducted using R software.

Results and Discussion

Effect of interaction between temperature and sugar on radial growth, fresh weight and dry weight of *Trichoderma asperellum*

The radial mycelial growth of *T. asperellum* exhibited considerable variation in response to different combinations of temperature and sugar concentration. Statistical analysis showed that these treatment interactions significantly affected fungal growth. The

maximum radial growth was recorded at 34 °C with a sugar concentration of 30 g/L, measuring a mean growth of 8.80 cm. This was followed by 30 °C with 20 g/L sugar, measuring a mean growth of 8.78 cm, while the least growth was recorded at 26 °C with 15 g/L sugar, measuring 4.72 cm (Table 2).

The results reveal that the radial growth of the different isolates of *T. asperellum* was significantly affected by all the combinations of temperature and sugar concentration. TA3 had the highest radial growth of 9.00 cm at 20 g/L sugar concentration. Similarly, isolate TA4 had a mean radial growth of 9.00 cm at 30 g/L sugar concentration. Isolates TA1 and TA2 also had relatively high radial growth of 7.55 cm and 7.47 cm respectively, under specific temperature and sugar concentrations. The radial growth of all isolates ranged between 2.50 cm and 9.00 cm depending on the combination of temperature and sugar concentration (Fig. 1).

The result of the interaction of the different temperatures and sugar concentrations showed that the maximum mycelial fresh weight was obtained at 30 °C with a sugar concentration of 25 g/L, registering a mean weight of 5.45 g. This was followed by 30 °C with a sugar concentration of 10 g/L, registering a mean fresh weight of 4.69 g. The lowest fresh weight was obtained at 28 °C with a sugar concentration of 20 g/L, registering a mean of 3.37 g (Table 3).

The experiment also showed that the fresh weight of mycelia was significantly different among the various isolates of *T. asperellum* for all temperature and sugar concentrations. Isolate TA3 had the highest mean fresh weight for all treatment combinations, with a mean of 4.53 g. In addition, isolates TA2, TA3 and TA4 had significantly higher fresh weights for different temperature and sugar concentrations. Based on the density graph (Fig. 2), the fresh weight of mycelia for all isolates ranged from 2.50 g to 5.90 g depending on the treatment concentrations.

The interaction of 30 °C temperature and 10 g/L sugar concentration was determined to be optimal for the maximum mycelial dry weight. Under this treatment concentration, the maximum mean dry weight was 0.58 g, followed by 0.56 g at 34 °C and 20 g/L sugar concentration. The lowest mycelial dry weight was recorded at 28 °C and 30 g/L sugar concentration, with a mean of 0.38 g (Table 4).

The study also revealed that different *T. asperellum* isolates showed significant variations in dry weight across all temperature and sugar concentration combinations. Isolate TA3 showed the highest mean dry weight value consistently in all treatments, with an average of 0.52 g. Moreover, isolates TA2, TA3 and TA4 exhibited better dry weight values in all combinations of temperature and sugar concentrations.

From the density plot (Fig. 3), the mycelial dry weight of all isolates varied between 0.25 g and 0.82 g based on the treatment conditions. However, a temperature of 26 °C and low sugar concentration of 15 g/L was not ideal for the isolates' growth. These findings are consistent with previous researchers who established that *Trichoderma* grew optimally at 25 °C (23) and that *Trichoderma harzianum* grows best with glucose concentrations ranging between 1.5 to 2.5 % (24).

The study concludes that temperature and sugar concentration had a big effect on *T. asperellum* radial growth, fresh weight and dry weight. Maximum radial growth at 30–34 °C with 20–30 g/L sugar indicates that higher temperature and adequate carbon supply enhance fungal metabolism, enzymatic activity and

Table 2. Effect of interaction between different temperature and sugar concentration on the radial growth of mycelium of *Trichoderma asperellum*

Sugar (g/L) Temperature (°C)	Radial growth (cm)															Mean										
	10					15					20						25					30				
	26	28	30	32	34	26	28	30	32	34	26	28	30	32	34		26	28	30	32	34	26	28	30	32	34
TA1	6.62 (0.88)	7.42 (0.93)	6.74 (0.89)	9.00 (1.00)	4.46 (0.74)	4.38 (0.73)	9.00 (1.00)	6.18 (0.86)	6.90 (0.90)	9.00 (1.00)	6.50 (0.88)	9.00 (1.00)	8.20 (0.96)	9.00 (1.00)	8.26 (0.97)	3.18 (0.62)	6.12 (0.85)	8.10 (0.96)	9.00 (1.00)	8.18 (0.96)	7.55 (0.92)					
	9.00 (1.00)	6.42 (0.87)	6.34 (0.87)	6.42 (0.87)	7.34 (0.92)	2.74 (0.57)	3.46 (0.65)	9.00 (1.00)	9.00 (1.00)	6.86 (0.90)	7.06 (0.91)	9.00 (1.00)	9.00 (1.00)	9.00 (1.00)	7.78 (0.94)	3.12 (0.61)	9.00 (1.00)	8.16 (0.96)	3.94 (0.69)	9.00 (1.00)	7.47 (0.91)					
TA2	5.06 (0.78)	4.46 (0.74)	4.66 (0.75)	4.50 (0.74)	4.54 (0.74)	9.00 (1.00)	7.66 (0.94)	9.00 (1.00)	9.00 (1.00)	7.36 (0.92)	9.00 (1.00)	9.00 (1.00)	4.60 (0.75)	3.98 (0.70)	3.60 (0.66)	9.00 (1.00)	9.00 (1.00)	9.00 (1.00)	4.54 (0.74)	9.00 (1.00)	6.89 (0.88)					
	5.28 (0.80)	9.00 (1.00)	9.00 (1.00)	9.00 (1.00)	9.00 (1.00)	2.74 (0.57)	8.18 (0.96)	9.00 (1.00)	5.10 (0.79)	4.14 (0.71)	6.14 (0.85)	7.72 (0.94)	6.84 (0.89)	5.64 (0.82)	3.44 (0.65)	9.00 (1.00)	9.00 (1.00)	9.00 (1.00)	9.00 (1.00)	7.30 (0.90)						
TA3	6.49 (0.87)	6.83 (0.88)	6.69 (0.88)	7.23 (0.90)	6.34 (0.85)	4.72 (0.72)	7.08 (0.89)	8.30 (0.96)	7.50 (0.92)	6.84 (0.88)	8.20 (0.96)	7.57 (0.92)	7.36 (0.91)	6.91 (0.88)	5.77 (0.80)	6.08 (0.81)	8.28 (0.96)	8.57 (0.98)	6.62 (0.86)	8.80 (0.99)						
TA4																										
Mean																										
Isolates																										
Sugar																										
Temperature																										
Isolates × Sugar																										
Isolates × Temperature																										
Sugar × Temperature																										
Isolates × Sugar × Temperature																										

Table 3. Effect of interaction between different temperature and sugar concentration on the mycelium fresh weight of *Trichoderma asperellum*

Sugar (g/L) Temperature (°C)		Mycelial fresh weight (g)														Mean								
		10				15				20				25			30							
		26	28	30	32	34	26	28	30	32	34	26	28	30	32		34	26	28	30	32	34		
TA1	3.50 (0.65)	3.21 (0.62)	3.24 (0.63)	3.50 (0.65)	4.15 (0.71)	4.40 (0.73)	3.16 (0.62)	4.19 (0.72)	4.13 (0.71)	4.25 (0.72)	2.66 (0.56)	2.79 (0.58)	2.87 (0.59)	3.00 (0.60)	3.71 (0.67)	4.43 (0.73)	2.74 (0.57)	5.90 (0.84)	4.27 (0.72)	3.08 (0.61)	3.23 (0.63)	4.12 (0.71)	4.06 (0.70)	4.02 (0.70)
	4.10 (0.71)	4.35 (0.73)	5.64 (0.82)	4.23 (0.72)	3.81 (0.68)	4.05 (0.70)	4.50 (0.74)	4.63 (0.75)	4.20 (0.72)	4.40 (0.73)	2.74 (0.57)	2.94 (0.59)	3.08 (0.61)	3.18 (0.62)	3.03 (0.61)	4.63 (0.75)	4.22 (0.72)	5.41 (0.81)	4.49 (0.74)	4.21 (0.72)	3.43 (0.65)	4.42 (0.73)	4.43 (0.73)	4.29 (0.72)
TA2	4.52 (0.74)	4.12 (0.71)	5.49 (0.81)	4.49 (0.74)	4.53 (0.74)	4.78 (0.76)	4.63 (0.75)	4.81 (0.76)	4.48 (0.74)	4.49 (0.74)	4.90 (0.77)	4.34 (0.73)	4.13 (0.71)	3.43 (0.65)	5.03 (0.78)	4.70 (0.76)	4.68 (0.75)	5.24 (0.80)	4.49 (0.74)	4.35 (0.73)	4.56 (0.75)	4.70 (0.76)	4.59 (0.75)	4.24 (0.72)
	3.68 (0.67)	3.48 (0.65)	4.39 (0.73)	4.10 (0.71)	4.23 (0.72)	4.37 (0.73)	4.71 (0.76)	4.81 (0.76)	5.47 (0.81)	4.70 (0.75)	3.54 (0.66)	3.41 (0.64)	4.20 (0.72)	3.95 (0.69)	4.91 (0.77)	4.76 (0.76)	4.76 (0.76)	5.25 (0.80)	3.91 (0.69)	4.20 (0.72)	4.66 (0.75)	4.10 (0.71)	4.40 (0.73)	4.21 (0.72)
TA3	3.95 (0.69)	3.79 (0.68)	4.69 (0.75)	4.08 (0.70)	4.18 (0.71)	4.40 (0.73)	4.25 (0.72)	4.61 (0.75)	4.57 (0.74)	4.46 (0.74)	3.46 (0.64)	3.37 (0.64)	3.57 (0.66)	3.39 (0.64)	4.17 (0.71)	4.63 (0.75)	4.10 (0.70)	5.45 (0.81)	4.29 (0.72)	3.96 (0.69)	3.97 (0.69)	3.65 (0.67)	4.41 (0.73)	4.19 (0.72)
	SED														CD 5 %									
Isolates	0.01100														0.02162									
	0.01230														0.02417									
Sugar	0.01230														0.02417									
	0.02459														0.04835									
Temperature	0.02459														0.04835									
	0.02750														0.05406									
Isolates × Sugar	0.05499														0.10811									

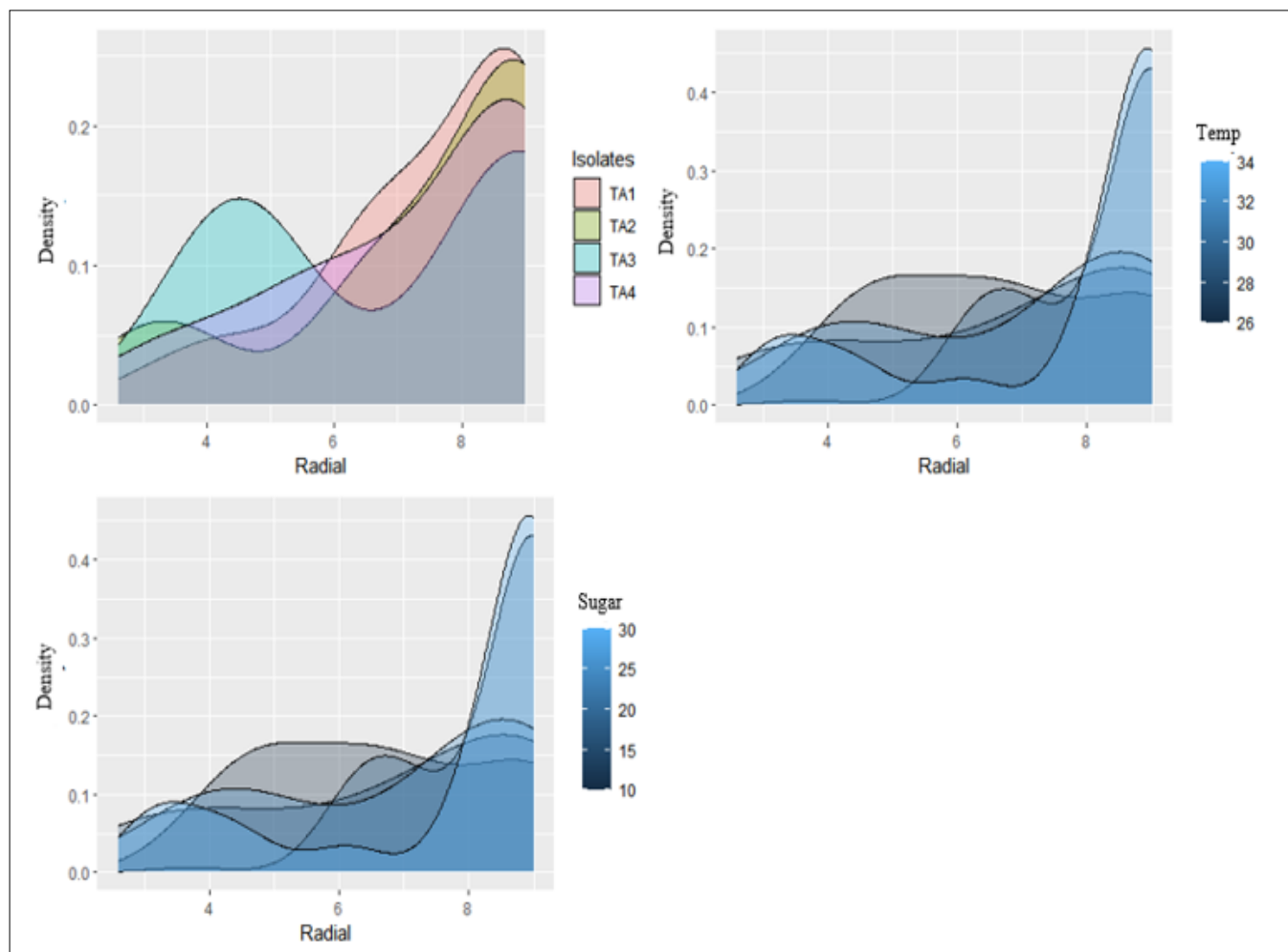


Fig. 1. Density graph shows radial growth of isolates of *Trichoderma asperellum* isolates at different temperatures and sugar concentration.

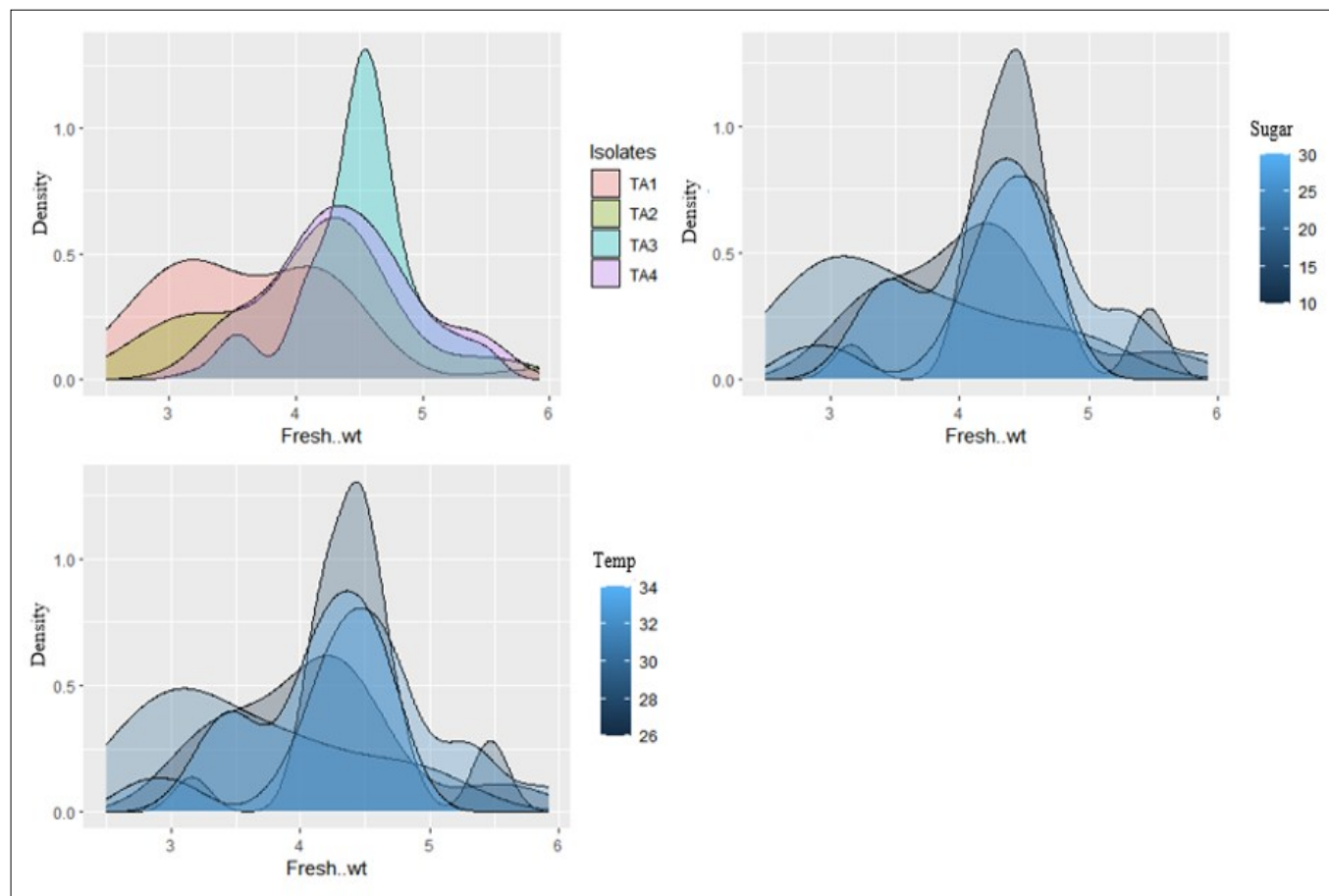


Fig. 2. Density graph shows mycelial fresh weight of isolates of *Trichoderma asperellum* isolates at different temperatures and sugar concentration.

Table 4. Effect of interaction between different temperature and sugar concentration on the mycelium dry weight of *Trichoderma asperellum*

[illegible]

hyphal extension (25). Similar results were reported that *Trichoderma* grew best at 28–32 °C. Higher sugar concentrations enhanced biomass production by augmenting respiration and the synthesis of cellular components. Similar findings shows that *T. harzianum* grows best at 1.5–2.5 % glucose (24). The highest fresh weight at 30 °C with 25 g/L sugar indicates that moderate temperature and adequate carbon promote mycelial biomass, while lower sugar levels and temperature inhibit growth. Similar findings, demonstrating that elevated carbon concentration substantially enhanced the mycelial biomass of *Trichoderma* species under regulated temperature conditions (26). The lower fresh weight seen at suboptimal temperature combinations suggests that enzymes are less effective and nutrients are absorbed more slowly.

The dry weight was highest at moderate sugar levels (10–20 g/L), which means that carbon was turned into structural biomass efficiently. Too much sugar, on the other hand, made the moisture content higher and the dry matter lower. Similar observations were also reported where biocontrol agent was effective against *Sclerotium rolsii* and *Rhizoctonia solani* (27). The differences between isolates, with TA3 and TA4 doing better, show that there is physiological diversity in how nutrients are used, as found by the scientist where they showed the mechanism of growth of *Trichoderma spp* (7). In general, *T. asperellum* grows and makes more biomass best at temperatures between 30 °C and 34 °C. This is important for mass multiplication.

Conclusion

The reaction of temperature and sugar concentration influenced the growth of *Trichoderma asperellum* significantly. The findings demonstrated that the higher the temperature the greater the growth of the fungus was in the presence of some levels of sugar. Temperature was a major aspect in modulation of biological activity, and it had a major impact on the radial growth of *T. asperellum* which grew within the range of 26–30 °C. High temperature has been attributed to the capability of the fungus to multiply, which is attributed to the high level of sugar generation, including trehalose, mannose and raffinose. The concentration of sugar also played a significant role in the growth of the fungus and *T. asperellum* grew well at temperatures of 30 °C and 34 °C and sugars of concentration of 15 to 30 g/L. This study had a conclusion that isolates of *T. asperellum* could grow in a broad temperature and sugar concentration. Future research should evaluate the performance of *T. asperellum* under field conditions. Additionally, studies focusing on optimisation of formulation and shelf life under varying environmental conditions will help in developing effective commercial applications.

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Authors' contributions

MMRA carried out the experiment, took observations and analysed the data. TMN guided the research by formulating the research

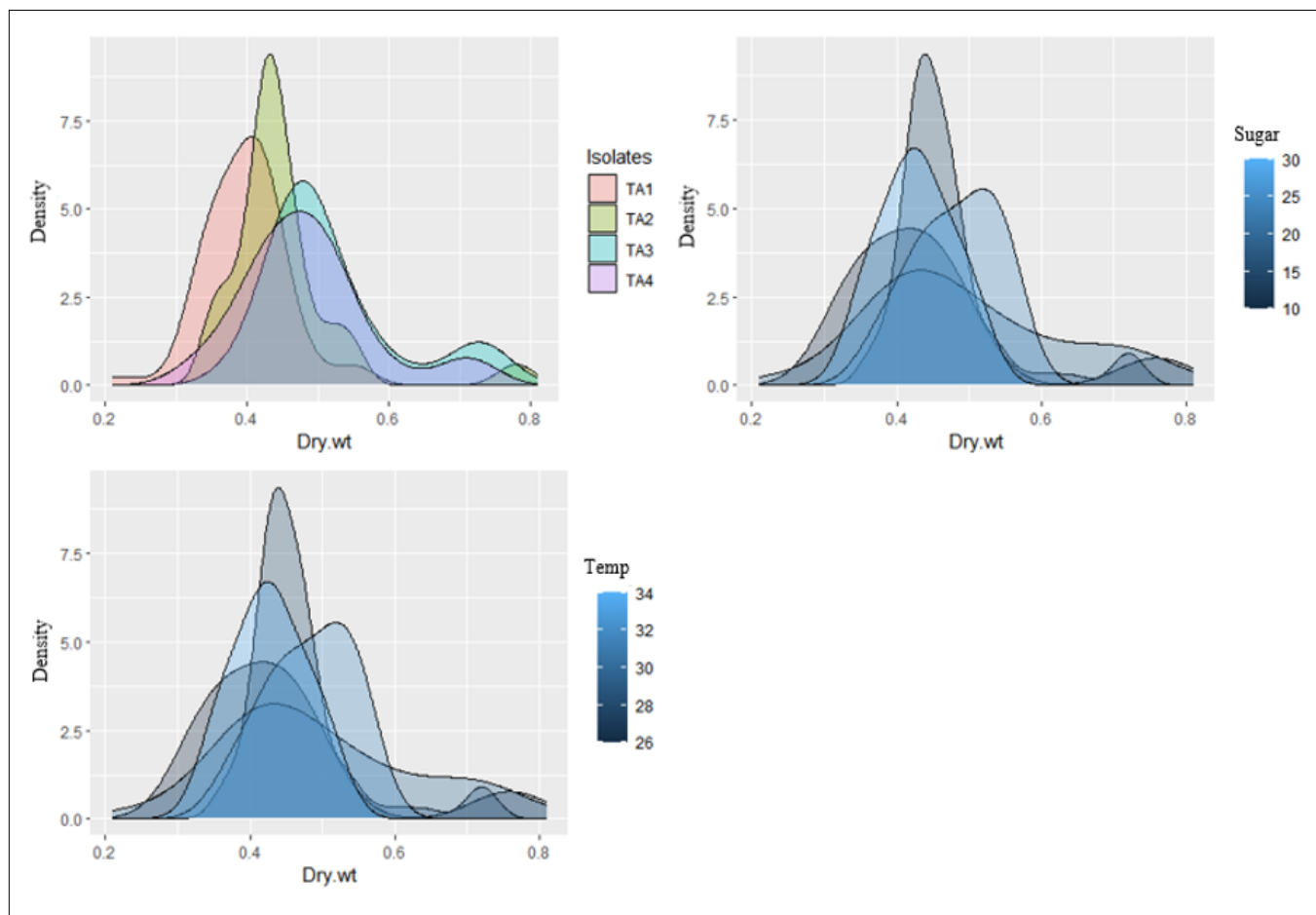


Fig. 3. Density graph shows mycelial dry weight of isolates of *Trichoderma asperellum* isolates at different temperature and sugar concentration.

concept and RJ, LNS, PK approved the final manuscript. MCL, YAS, BT, SD participated in the design of the study and performed the statistical analysis. GG, NM, SKS and BS contributed by imposing the experiment and helped to edit, summarise, and revise the manuscript. TH, IG, FAK, SMI and BS helped to summarise and revise the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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